stilbene, imipramine, indomethacin, isocarbostyril, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 12 μm Dynamax C18 (Rainin)
Mobile phase: pH 7.5 sodium phosphate buffer
Flow rate: 2
Detector: UV 266

CHROMATOGRAM

Retention time: 5.4

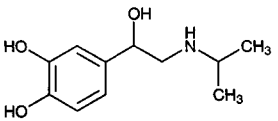
OTHER SUBSTANCES

Simultaneous: metabolites, acetylisoniazid

REFERENCE

Hickman,D.; Palamanda,J.R.; Unadkat,J.D.; Sim,E. Enzyme kinetic properties of human recombinant arylamine N-acetyltransferase 2 allotypic variants expressed in *Escherichia coli*, *Biochem.Pharmacol.*, **1995**, *50*, 697–703.

Isoproterenol



Molecular formula: $C_{11}H_{17}NO_3$
Molecular weight: 211.26
CAS Registry No.: 7683-59-2, 51-30-9 (HCl), 6700-39-6 (sulfate dihydrate), 299-95-6 (sulfate)
Merck Index: 5236

SAMPLE

Matrix: blood

Sample preparation: Plasma. Prepare a SPE column by adding 500 μL of a 20% suspension of 19-40 μm Toyopak SP (strong cation-exchange sulfopropyl resin, Na^+ (Toyo Soda)) in water to a 35 × 6 column, wash with two 1 mL portions of 2 M LiOH, wash with two 5 mL portions of water, wash with two 1 mL portions of EtOH:12 M HCl 90:10, wash with two 5 mL portions of water, wash with three 1 mL portions of buffer. 500 μL Plasma + 500 μL buffer, mix, add to the SPE column, wash with two 5 mL portions of water, wash with 1 mL MeCN:water 50:50, elute with 300 μL 600 μM potassium ferricyanide in 600 mM KCl:MeCN 50:50, add 50 μL reagent to the eluate, heat at 37° for 40 min, cool in ice-water, inject a 100 μL aliquot. Urine. 10 μL Urine + 1 mL MeCN:500 mM KCl 60:40 + 10 μL 75 mM potassium hexacyanoferrate(III) + 100 μL reagent, heat at 37° for 40 min, inject a 100 μL aliquot (J. Chromatogr. 1986, 380, 229). (Prepare buffer by mixing 8 volumes 250 mM LiOH in 200 mM phosphoric acid with 1 volume 200 mM phosphoric acid, pH 5.8. Prepare reagent by dissolving 212 mg 1,2-diphenylethylenediamine in 10 mL 100 mM HCl, pH 6.7.)

HPLC VARIABLES

Column: 150 × 4.6 5 μm TSK-gel ODS-120T (Toyo Soda)
Mobile phase: MeCN:MeOH:50 mM pH 7.0 Tris-HCl buffer 50:10:40 (Wash with MeCN:MeOH:water 50:10:40 for 15 min at the end of each day.)
Flow rate: 1
Injection volume: 100
Detector: F ex 345 em 485 (plasma), F ex 350 em 480 (urine)

CHROMATOGRAM

Retention time: 8
Internal standard: isoproterenol

OTHER SUBSTANCES

Extracted: dopamine, epinephrine, norepinephrine

KEY WORDS

derivatization; plasma; SPE; isoproterenol is IS

REFERENCE

Mitsui,A.; Nohta,H.; Ohkura,Y. High-performance liquid chromatography of plasma catecholamines using 1,2-diphenylethylenediamine as precolumn fluorescence derivatization reagent, *J.Chromatogr.*, **1985**, *344*, 61–70.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 250 μL 1 ng/mL α-methylnorepinephrine + 1 mL buffer + 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol, shake for 2 min, centrifuge at 20° at 1000 g for 5 min, freeze in acetone/dry ice. Remove the organic phase and add it to 2 mL 1-octanol and 200 μL 80 mM acetic acid, shake, centrifuge at 20° at 1000 g for 5 min, freeze in acetone/dry ice. Discard the organic phase, thaw the aqueous phase and add it to 1 mL 10 mM HCl, 1 mL buffer, and 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol, shake for 2 min, centrifuge at 20° at 1000 g for 5 min, freeze in acetone/dry ice. Remove the organic phase and add it to 2 mL 2 M pH 8.6 ammonia/ammonium chloride buffer containing 13.4 mM EDTA, shake, freeze in dry ice/acetone. Remove the organic layer and add it to 2 mL 1-octanol and 150 μL 80 mM acetic acid, shake, centrifuge at 20° at 1000 g for 5 min, freeze in dry ice/acetone, discard the organic layer. Thaw the aqueous layer and add it to 250 μL MeCN, 50 μL 1.75 M pH 7.05 bicine, and 100 μL 100 mM 1,2-diphenylethylenediamine in 100 mM HCl, add 20 μL 20 mM potassium ferricyanide in water, heat at 37° in the dark for 1 h, keep at 20° in the dark, inject a 100 μL aliquot. (Buffer was 2 M pH 8.6 ammonia/ammonium chloride buffer containing 8.9 mM diphenylborate-ethanolamine complex and 13.4 mM EDTA. Stir buffer with 45 g/L activated alumina for 2 h before use. Wash 1-octanol with 80 mM acetic acid. Recrystallize 1,2-diphenylethylenediamine from toluene:light petroleum (bp 60-80°) 10:90, dry overnight at 60°.)

HPLC VARIABLES

Column: 100 × 4.6 3 μm Cp MicroSpher C18 (Chrompack)
Mobile phase: MeCN:MeOH:50 mM pH 7.0 sodium acetate buffer 40:8:50
Flow rate: 1
Injection volume: 100

Detector: F ex 350 em 480

CHROMATOGRAM
Retention time: 8
Internal standard: α-methylnorepinephrine (3)

OTHER SUBSTANCES
Extracted: dihydroxybenzylamine, dopamine, epinephrine, norepinephrine

KEY WORDS
plasma; derivatization; comparison with electrochemical detection

REFERENCE
van der Hoorn,F.A.J.; Boomsma,F.; Man in 't Veld,A.J.; Schalekamp,M.A.D.H. Determination of catecholamines in human plasma by high-performance liquid chromatography: comparison between a new method with fluorescence detection and an established method with electrochemical detection, *J.Chromatogr.*, **1989**, *487*, 17–28.

SAMPLE
Matrix: blood
Sample preparation: Plasma. 1 mL Plasma + 1 mL buffer + 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol, shake for 2 min, centrifuge at 1000 g for 5 min, freeze in dry ice/acetone. Remove the organic phase and add it to 2 mL 1-octanol (saturated with 80 mM acetic acid) and 200 μL 80 mM acetic acid, shake, centrifuge at 1000 g for 5 min. Freeze the aqueous layer and remove the organic layer. Add 1 mL 10 mM HCl, 1 mL buffer, and 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol to the aqueous phase. Shake, centrifuge, freeze, remove the organic layer and add it to 2 mL 2 M pH 8.6 ammonia-ammonium chloride buffer containing 13.4 mM EDTA (but no complex). Freeze, remove the organic layer and add it to 2 mL 1-octanol and 150 μL 80 mM acetic acid, shake, centrifuge at 1000 g for 5 min. Freeze, remove the organic layer and add the aqueous layer to 200 μL MeCN, 50 μL 1.75 M pH 6.95 bicine buffer containing 1% EDTA, 100 μL 100 mM 1,2-diphenylethylenediamine in 100 mM HCl, and 20 μL 20 mM potassium ferricyanide in water. Heat at 37° in the dark for 1 h, inject a 50 μL aliquot (keep it in the dark in the autosampler). Urine. 100 μL Urine + 1 mL 10 mM HCl + 1 mL buffer + 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol, shake for 2 min, centrifuge at 1000 g for 5 min, freeze in dry ice/acetone. Remove the organic phase and add it to 2 mL 1-octanol (saturated with 80 mM acetic acid) and 200 μL 80 mM acetic acid, shake, centrifuge at 1000 g for 5 min. Freeze the aqueous layer and remove the organic layer. Add 1 mL 10 mM HCl, 1 mL buffer, and 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol to the aqueous phase. Shake, centrifuge, freeze, remove the organic layer and add it to 2 mL 1-octanol and 150 μL 80 mM acetic acid, shake, centrifuge at 1000 g for 5 min. Freeze, remove the organic layer and add the aqueous layer to 200 μL MeCN, 50 μL 1.75 M pH 6.95 bicine buffer containing 1% EDTA, 100 μL 100 mM 1,2-diphenylethylenediamine in 100 mM HCl, and 20 μL 20 mM potassium ferricyanide in water. Heat at 37° in the dark for 1 h, inject a 20 μL aliquot (keep it in the dark in the autosampler). (Buffer was a 2 M pH 8.6 ammonia-ammonium chloride buffer containing 8.9 mM diphenyl borate-ethanolamine complex and 13.4 mM EDTA.)

HPLC VARIABLES
Column: 100 × 4.6 3 μm Spherisorb ODS2
Mobile phase: Gradient. A was MeCN:MeOH:50 mM pH 7.0 sodium acetate buffer 20:20:60. B was MeCN:MeOH:50 mM pH 7.0 sodium acetate buffer 60:10:30. A:B 52:48 for 6 min, go to 0:100 over 0.1 min, stay at 0:100 for another 10 min. Equilibrate at initial conditions for 4 min before next sample.
Flow rate: 1
Injection volume: 20-50
Detector: F ex 350 em 480

CHROMATOGRAM
Retention time: 9
Internal standard: isoproterenol

OTHER SUBSTANCES
Simultaneous: dobutamine, epinephrine, dopamine, epinine, norepinephrine, metabolites
Interfering: α-methyldopa

KEY WORDS
plasma; isoproterenol is IS; derivatization

REFERENCE
Alberts,G.; Boomsma,F.; Man in 't Veld,A.J.; Schalekamp,M.A.D.H. Simultaneous determination of catecholamines and dobutamine in human plasma and urine by high-performance liquid chromatography with fluorimetric detection, *J.Chromatogr.*, **1992**, *583*, 236–240.

SAMPLE
Matrix: blood
Sample preparation: Mix plasma and N-methyldopamine, add to a TOYOPAK SP strong cationic exchange SPE cartridge (Tosoh), elute with MeCN:600 mM KCl 50:50 containing 0.6 mM potassium hexacyanoferrate (III), derivatize eluate with 1,2-diphenylethylenediamine, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 Nucleosil 5C18
Mobile phase: MeOH:50 mM Tris-HCl buffer 80:20, adjusted to pH 7.0
Flow rate: 1
Detector: F

KEY WORDS
plasma; guinea pig; SPE; derivatization; pharmacokinetics

REFERENCE
Ohtani,H.; Yamamoto,K.; Sawada,Y.; Iga,T. Antibronchospasmic, tachycardiac, and hypokalaemic effects of L-isoproterenol in guinea-pigs, *Biopharm.Drug Dispos.*, **1995**, *16*, 745–753.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a Toyopak IC-SP S sulfopropyl resin, H^+ form, SPE cartridge (Tosoh) with 10 mL water and 2 mL 200 mM pH 5.0 sodium phosphate buffer. Plasma. 700 μL Plasma + 50 μL 7 μM 3,4-dihydroxyphenylpropanoic acid + 350 μL 2 M perchloric acid, mix, centrifuge at 4° at 1000 g for 15 min. Remove a 700 μL aliquot of the supernatant and adjust the pH to 1.5-2.0 with about 150 μL 2 M potassium carbonate, centrifuge at 4° at 1000 g for 5 min, add the supernatant to the SPE cartridge, wash with 10 mL water, elute with 300 μL MeOH:2 M sodium perchlorate 7:93, filter (cellulose acetate membrane), inject a 100 μL aliquot of the filtrate. Urine. Collect human urine for 24 h in the presence of 10 mL 6 M HCl. 500 μL Urine + 25 μL 800 μM 3,4-dihydroxyphenylpropanoic acid + 500 μL 1 M perchloric acid, mix, centrifuge at 4° at 1000 g for 15 min. Remove a 700 μL aliquot of the supernatant and adjust the pH to 1.5-2.0 with about 130 μL 2 M potassium carbonate, centrifuge at 4° at 1000 g for 5 min, add the supernatant to the SPE cartridge, wash with 1.5 mL water, wash with 500 μL EtOH:water 50:50, wash with 5 mL water, elute with 500 μL 1.5 M KCl in MeOH:100 mM HCl 7:93, filter (cellulose acetate membrane), inject a 100 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm TSK-gel ODS-80TM (Tosoh)
Mobile phase: MeOH:buffer 7:93 (Buffer was 30 mM pH 2.5 citrate buffer containing 0.4 mM sodium octanesulfonate.)
Flow rate: 0.8
Injection volume: 100
Detector: F ex 350 em 480 following post-column reaction. The column effluent mixed with reagent A pumped at 0.3 mL/min and the mixture flowed through a 3 m × 0.5 mm ID stainless steel coil at 90°. The effluent from this coil mixed with reagent B pumped at 0.3 mL/min and the mixture flowed through a 10 m × 0.5 mm ID stainless steel coil at 90° and through a 1 m × 0.5 mm ID stainless steel cooling coil to the detector (Anal. Sci. 1991, 7, 257). (Reagent A was 10 mM sodium periodate containing 3 mM potassium ferricyanide. Reagent B was 30 mM

meso-1,2-diphenylethylenediamine in EtOH:water 70:30 containing 130 mM sodium methylate.)

CHROMATOGRAM
Retention time: 60
Internal standard: isoproterenol

OTHER SUBSTANCES
Extracted: dopamine, epinephrine, levodopa, metanephrine, 3-methoxytyramine, norepinephrine, normetanephrine

KEY WORDS
post-column reaction; plasma; SPE; isoproterenol is IS

REFERENCE
Jeon,H.-K.; Nohta,H.; Ohkura,Y. High-performance liquid chromatographic determination of catecholamines and their precursor and metabolites in human urine and plasma by postcolumn derivatization involving chemical oxidation followed by fluorescence reaction, *Anal.Biochem.*, **1992**, *200*, 332–338.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Dissolve powdered tablets in 10 mM HCl, filter if necessary, inject an aliquot. Injections, solutions. Dilute with 10 mM HCl, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Partisil-5 ODS-3
Mobile phase: MeOH:buffer 30:70 (Buffer was 10 mM sodium 1-octanesulfonate in 0.2% acetic acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 16.5
Limit of detection: 53 ng

OTHER SUBSTANCES
Simultaneous: norepinephrine, epinephrine, levonordefrin, phenylephrine, metaraminol, impurities

KEY WORDS
tablets; injections; ophthalmic solutions; inhalation solutions

REFERENCE
Smela,M.J.,Jr.; Stromberg,R. Liquid chromatographic determination of six sympathomimetic drugs in dosage forms, *J.Assoc.Off.Anal.Chem.*, **1991**, *74*, 289–291.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Grind tablets, weigh out a portion, dissolve in 50 mL mobile phase, sonicate, filter (No. 4 sintered glass plate), dilute, inject an aliquot. Capsules. Dissolve 10 capsules (without opening) in 100 mL mobile phase, sonicate, inject an aliquot. Injections, ampules, sprays. Dilute, inject an aliquot.

HPLC VARIABLES
Column: 120 × 4.6 Spherisorb C18 ODS-2
Mobile phase: Isopropanol:buffer 5:95 (Buffer was 100 mM sodium dodecyl sulfate containing 25 mM Na_2HPO_4, pH adjusted to 3.0 with HCl.)
Flow rate: 1
Injection volume: 20

Detector: UV 280

CHROMATOGRAM
Retention time: 6
Limit of detection: 16 ng/mL

OTHER SUBSTANCES
Simultaneous: carbidopa, dopamine, epinephrine, hydrochlorothiazide, levodopa, methyldopa, norepinephrine, phenylephrine

KEY WORDS
tablets; capsules; injections; ampules; sprays

REFERENCE
Villanueva Camañas,R.M.; Sanchis Mallols,J.M.; Torres Lapasió,J.R.; Ramis-Ramos,G. Analysis of pharmaceutical preparations containing catecholamines by micellar liquid chromatography with spectrophotometric detection, *Analyst*, **1995**, *120*, 1767–1772.

SAMPLE
Matrix: perfusate
Sample preparation: 30 μL Perfusate (artificial CSF) + 10 μL 200 mM perchloric acid. Mix a 25 μL aliquot with 12.5 μL reagent, let stand for 2 min, inject an aliquot. (Prepare a stock solution by dissolving 27 mg o-phthalaldehyde in 1 mL MeOH, add 5 μL β-mercaptoethanol, add 9 mL 100 mM pH 9.3 sodium tetraborate containing 10 μM EDTA. This solution is good for 5 days in a sealed amber bottle at room temperature. Prepare the working reagent by diluting 1 mL of the stock solution with 3 mL 100 mM pH 9.3 sodium tetraborate containing 10 μM EDTA, allow to stand for 24 h before use.)

HPLC VARIABLES
Column: two columns 150 × 4.6 5 μm M.S. Gel C18 (ESA)
Mobile phase: MeOH:buffer 8:92 adjusted to pH 3.0 with phosphoric acid (Buffer was 54 mM NaH_2PO_4 containing 1.24 mM sodium heptanesulfonate.)
Column temperature: 33
Flow rate: 1.2
Detector: E, ESA Coulochem Electrode Array System Model 5500, detector temp 33°, oxidation potential 70 mV

CHROMATOGRAM
Retention time: 8.90
Limit of quantitation: 0.5 ng/mL

OTHER SUBSTANCES
Extracted: apomorphine, dopamine, hydralazine, methoxamine, morphine, norepinephrine, phenylephrine

KEY WORDS
rat; derivatization

REFERENCE
Acworth,I.N.; Yu,J.; Ryan,E.; Gariepy,K.C.; Gamache,P.; Hull,K.; Maher,T. Simultaneous measurement of monoamine, amino acid, and drug levels, using high performance liquid chromatography and coulometric array technology: application to in vivo microdialysis perfusate analysis, *J.Liq.Chromatogr.*, **1994**, *17*, 685–705.

SAMPLE
Matrix: solutions
Sample preparation: Dilute with 5% dextrose, inject a 20 μL aliquot.

HPLC VARIABLES
Column: Waters microparticulate C18
Mobile phase: MeOH:350 mM acetic acid and 5 mM sodium heptanesulfonate 35:65

Flow rate: 1.6-2.0
Injection volume: 20
Detector: F ex 285 em 315

CHROMATOGRAM
Retention time: 4.33

OTHER SUBSTANCES
Simultaneous: theophylline, terbutaline
Interfering: methyldopate

REFERENCE
Williams,D.A.; Fung,E.Y.Y.; Newton,D.W. Ion-pair high-performance liquid chromatography of terbutaline and catecholamines with aminophylline in intravenous solutions, *J.Pharm.Sci.*, **1982**, *71*, 956–958.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: Acetic acid:triethylamine:water 1.5:0.5:98
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM
Retention time: k' 0.71

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 cellulose tris(3,5-dimethylphenylcarbamate)
Mobile phase: Hexane:isopropanol:trichloroacetic acid 80:15:5
Flow rate: 0.5
Detector: UV

CHROMATOGRAM
Retention time: k' 1.12 (of first (+) enantiomer)

KEY WORDS
chiral; α 1.28

REFERENCE
Okamoto,Y.; Aburatani,R.; Hatano,K.; Hatada,K. Optical resolution of racemic drugs by chiral HPLC on cellulose and amylose tris(phenylcarbamate) derivatives, *J.Liq.Chromatogr.*, **1988**, *11*, 2147–2163.

SAMPLE
Matrix: solutions
Sample preparation: 1 mL Saline solution + 100 μL ice-cold 5 M acetic acid + 10 μL 270 mM disodium EDTA, stir, inject an aliquot.

HPLC VARIABLES
Guard column: Guard-Pak CN (Waters)

Column: 150 × 3.9 5 μm Nova-Pak C18
Mobile phase: MeCN:buffer 9:91 adjusted to pH 3.6 with 8.7 M phosphoric acid (Buffer was 70 mM Na_2HPO_4 containing 5 mM sodium heptanesulfonate and 0.1 mM disodium EDTA.) (Recirculate mobile phase.)
Flow rate: 1
Detector: E, Waters Model 410, glassy carbon working electrode + 0.825 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 4.2
Limit of detection: 0.1 pmole

OTHER SUBSTANCES
Simultaneous: 3-O-methylisoprenaline

KEY WORDS
saline

REFERENCE
Bryan,L.J.; O'Donnell,S.R. Analysis of the O-methylated metabolites of isoprenaline, adrenaline and noradrenaline in physiological salt solutions by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1989**, *487*, 29–39.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Partisil ODS-3
Mobile phase: MeOH:buffer 30:70 (Buffer was 10 mM octanesulfonic acid in 0.2% acetic acid.)
Flow rate: 1
Detector: UV 220

CHROMATOGRAM
Retention time: 16.5

OTHER SUBSTANCES
Simultaneous: epinephrine, levonordefrin, metaraminol, phenylephrine

REFERENCE
Phenomenex Catalog, **1994**, p. 1.077.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Chirex 3020 (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 55:35:10 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 0.7-1
Injection volume: 20
Detector: UV 282

KEY WORDS

chiral; α = 1.21 for enantiomers

REFERENCE
Cleveland,T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J.Liq.Chromatogr.*, **1995**, *18*, 649–671.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Sumchiral CSP 10 (Sumika Chemical Analysis Service)
Mobile phase: n-Hexane:1,2-dichloroethane:MeOH:trifluoroacetic acid 250:140:20:1
Flow rate: 1
Detector: UV 230-280

CHROMATOGRAM
Retention time: 22 (+), 25 (-)

KEY WORDS
chiral

REFERENCE
Oi,N.; Kitahara,H.; Aoki,F. Direct enantiomer separations by high-performance liquid chromatography with chiral urea derivatives as stationary phases, *J.Chromatogr.A*, **1995**, *694*, 129–134.

SAMPLE
Matrix: solutions
Sample preparation: Swab surface with mobile phase, shake swab with mobile phase for 20 min, filter (0.20 μm PDVF membrane), inject a 50 μL aliquot of the filtrate.

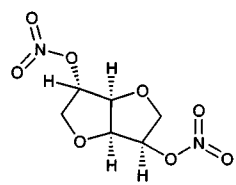
HPLC VARIABLES
Column: 150 × 4.6 5 μm Nucleosil C18
Mobile phase: MeOH:buffer 10:90 (Buffer was 50 mM KH_2PO_4 containing 5 mM sodium 1-pentanesulfonate and 100 μM disodium EDTA, pH adjusted to 3.6 with phosphoric acid.)
Flow rate: 1
Injection volume: 50
Detector: E, Bioanalytical Systems, thin-layer glassy carbon electrode +0.65 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 6.9
Limit of detection: 0.1 ng/mL

KEY WORDS
surface contamination

REFERENCE
Elrod,L.,Jr.; Schmit,J.L.; Morley,J.A. Determination of isoproterenol sulfate on surfaces using high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.A*, **1996**, *723*, 235–241.

Isosorbide dinitrate



Molecular formula: $C_6H_8N_2O_8$
Molecular weight: 236.14
CAS Registry No.: 87-33-2
Merck Index: 5245

SAMPLE
Matrix: blood

Sample preparation: 3 mL Plasma + 30 μL 1 μg/mL nitroglycerin in n-hexane + 12 mL dichloromethane:ethyl acetate 1:1, shake mechanically at 250 cycles/min for 5 min, centrifuge at 550 g at 4° for 5 min. Remove the organic phase and evaporate it to about 20 μL under a stream of nitrogen at room temperature, inject.

HPLC VARIABLES
Column: 250 × 4 10 μm Zorbax NH_2
Mobile phase: n-Hexane:MeOH 95:5
Flow rate: 5
Injection volume: 20
Detector: Thermal energy analyzer, Thermo Electron Corp. Model 502A, furnace temp 575°, argon 15 mL/min, oxygen 25 mL/min, MeOH/dry ice slush bath

CHROMATOGRAM
Retention time: 3.3
Internal standard: nitroglycerin (5.0)
Limit of detection: 0.25-0.5 ng/mL
Limit of quantitation: 0.56 ng/mL

OTHER SUBSTANCES
Simultaneous: isosorbide mononitrate

KEY WORDS
plasma

REFERENCE
Maddock,J.; Lewis,P.A.; Woodward,A.; Massey,P.R.; Kennedy,S. Determination of isosorbide dinitrate and its mononitrate metabolites in human plasma by high-performance liquid chromatography-thermal energy analysis, *J.Chromatogr.*, **1983**, *272*, 129–136.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Prepare a 500 μg/mL aqueous solution, filter, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 200 × 2.1 5 μm Hypersil ODS
Mobile phase: MeOH:water 20:80
Flow rate: 0.4 for 3 min, to 0.6 over 0.5 min, maintain at 0.6 for 9.5 min, return to 0.4 over 0.5 min
Injection volume: 5
Detector: UV 210

CHROMATOGRAM
Retention time: 10.55

OTHER SUBSTANCES
Simultaneous: isosorbide mononitrate
Noninterfering: lactose

REFERENCE
Azcona,T.; Martin-Gonzalez,A.; Zamorano,P.; Pascual,C.; Grau,C.; Garcia de Mirasierra,M. New methods for the assay of 5-isosorbide mononitrate and its validation, *J.Pharm.Biomed.Anal.*, **1991**, *9*, 725–729.

SAMPLE
Matrix: formulations
Sample preparation: Dissolve in water.

HPLC VARIABLES
Column: 300 × 3.9 μBondapack phenyl
Mobile phase: MeCN:THF:water 26:10:64

Flow rate: 2
Injection volume: 10-40
Detector: UV 218

CHROMATOGRAM
Retention time: 6
Internal standard: isosorbide dinitrate

OTHER SUBSTANCES
Simultaneous: nitroglycerin, isosorbide dinitrate is IS

KEY WORDS
injections

REFERENCE
Baaske,D.M.; Carter,J.E.; Amann,A.H. Rapid and accurate stability-indicating assay for nitroglycerin, *J.Pharm.Sci.*, **1979**, *68*, 481–483.

SAMPLE
Matrix: formulations
Sample preparation: Powder tablets, weigh out a portion equivalent to 1 mg isosorbide dinitrate, add to 10 mL 75 μg/mL nitroglycerin in MeOH, sonicate for 2 min, shake mechanically for 30 min, filter, inject an aliquot

HPLC VARIABLES
Guard column: 40 × 4.6 μBondapak C18/Corasil
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water 40:60
Flow rate: 1
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 9.5
Internal standard: nitroglycerin (14)

OTHER SUBSTANCES
Simultaneous: pentaerythritol tetranitrate, erythrityl tetranitrate

KEY WORDS
tablets

REFERENCE
Olsen,C.S.; Scroggins,H.S. High-performance liquid chromatographic determination of the nitrate esters isosorbide dinitrate, pentaerythritol tetranitrate, and erythrityl tetranitrate in various tablet forms, *J.Pharm.Sci.*, **1984**, *73*, 1303–1304.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out an amount of finely powdered tablets or capsules equivalent to about 25 mg of drug. Add 50 mL buffer, shake for 30 min, add 10 mL 5 mg/mL nitroglycerin in MeOH, make up to 100 mL with buffer, filter (0.45 μm), inject a 20 μL aliquot. If the sample clumps when the buffer is added, agitate with a stirring rod and sonicate. (Buffer was MeOH: 200 mM ammonium acetate buffer:water 55:10:35.)

HPLC VARIABLES
Guard column: 50 × 6.4 25-37 μm Whatman Co-Pell ODS
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeOH:200 mM ammonium acetate buffer:water 55:10:35
Flow rate: 1

Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 6
Internal standard: nitroglycerin (8)

OTHER SUBSTANCES
Simultaneous: isosorbide mononitrate, saccharin, pentaerythritol tetranitrate

KEY WORDS
tablets; capsules; stability-indicating

REFERENCE
Carlson,M.; Thompson,R.D.; Snell,R.P. Determination of isosorbide dinitrate in pharmaceutical products by HPLC, *J.Chromatogr.Sci.*, **1988**, *26*, 574–578.

SAMPLE
Matrix: formulations
Sample preparation: Inject directly.

HPLC VARIABLES
Column: 250 × 4.6 LiChrosorb 10 RP 8
Mobile phase: MeOH:water 50:50
Flow rate: 2
Injection volume: 10
Detector: UV 214

CHROMATOGRAM
Retention time: 3.8

OTHER SUBSTANCES
Simultaneous: nitroglycerin

KEY WORDS
saline

REFERENCE
Martens,H.J.; de Goede,P.N.; van Loenen,A.C. Sorption of various drugs in polyvinyl chloride, glass, and polyethylene-lined infusion containers, *Am.J.Hosp.Pharm.*, **1990**, *47*, 369–373.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: C18
Mobile phase: MeOH:water 50:50
Flow rate: 1.3
Detector: UV 220

CHROMATOGRAM
Internal standard: isosorbide dinitrate

OTHER SUBSTANCES
Simultaneous: nitroglycerin

KEY WORDS
injections; 5% dextrose; isosorbide dinitrate is IS

REFERENCE
Pramar,Y.; Das Gupta,V.; Gardner,S.N.; Yau,B. Stabilities of dobutamine, dopamine, nitroglycerin and sodium nitroprusside in disposable plastic syringes, *J.Clin.Pharm.Ther.*, **1991**, *16*, 203–207.

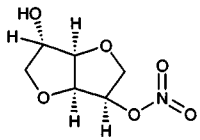
SAMPLE
Matrix: solutions
Sample preparation: 500 μL Buffer solution + 500 μL 1% trifluoroacetic acid, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 YMC R-ODS-1 5-ST
Mobile phase: MeCN:water 45:55 containing 0.05% trifluoroacetic acid
Flow rate: 1.1
Injection volume: 5
Detector: UV 230

KEY WORDS
buffer

REFERENCE
Fukuyama,S.; Hirasawa,Y.; Cox,D.; Koda,S.; Kita,Y. Acceleration of nitric oxide (NO) release from FK409, a spontaneous NO releaser, in the presence of sulfhydryl-bearing compounds, *Pharm.Res.*, **1995**, *12*, 1948–1952.

Isosorbide mononitrate



Molecular formula: $C_6H_9NO_6$
Molecular weight: 191.14
CAS Registry No.: 16051-77-7
Merck Index: 5245

SAMPLE
Matrix: blood
Sample preparation: 3 mL Plasma + 30 μL 1 μg/mL nitroglycerin in n-hexane + 12 mL dichloromethane:ethyl acetate 1:1, shake mechanically at 250 cycles/min for 5 min, centrifuge at 550 g at 4° for 5 min. Remove the organic phase and evaporate it to about 20 μL under a stream of nitrogen at room temperature, inject.

HPLC VARIABLES
Column: 250 × 4 10 μm Zorbax NH_2
Mobile phase: n-Hexane:MeOH 95:5
Flow rate: 5
Injection volume: 20
Detector: Thermal energy analyzer, Thermo Electron Corp. Model 502A, furnace temp 575°, argon 15 mL/min, oxygen 25 mL/min, MeOH/dry ice slush bath

CHROMATOGRAM
Retention time: 5.8 (2-isomer), 8.4 (5-isomer)
Internal standard: nitroglycerin (5.0)
Limit of detection: 1-1.2 ng/mL (5-isomer), 0.5-0.8 ng/mL (2-isomer)
Limit of quantitation: 1.66 ng/mL (5-isomer), 0.86 ng/mL (2-isomer)

OTHER SUBSTANCES
Simultaneous: isosorbide dinitrate

KEY WORDS
plasma

REFERENCE
Maddock,J.; Lewis,P.A.; Woodward,A.; Massey,P.R.; Kennedy,S. Determination of isosorbide dinitrate and its mononitrate metabolites in human plasma by high-performance liquid chromatography-thermal energy analysis, *J.Chromatogr.*, **1983**, *272*, 129–136.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Prepare a 500 μg/mL aqueous solution, filter, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 200 × 2.1 5 μm Hypersil ODS
Mobile phase: MeOH:water 20:80
Flow rate: 0.4 for 3 min, to 0.6 over 0.5 min, maintain at 0.6 for 9.5 min, return to 0.4 over 0.5 min
Injection volume: 5
Detector: UV 210

CHROMATOGRAM
Retention time: 3.06 (2 isomer), 3.49 (5 isomer)

OTHER SUBSTANCES
Simultaneous: isosorbide dinitrate
Noninterfering: lactose

REFERENCE
Azcona,T.; Martin-Gonzalez,A.; Zamorano,P.; Pascual,C.; Grau,C.; Garcia de Mirasierra,M. New methods for the assay of 5-isosorbide mononitrate and its validation, *J.Pharm.Biomed.Anal.*, **1991**, *9*, 725–729.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out an amount of finely powdered tablets or capsules equivalent to about 25 mg of drug. Add 50 mL buffer, shake for 30 min, add 10 mL 5 mg/mL nitroglycerin in MeOH, make up to 100 mL with buffer, filter (0.45 μm), inject a 20 μL aliquot. If the sample clumps when the buffer is added, agitate with a stirring rod and sonicate. (Buffer was MeOH: 200 mM ammonium acetate buffer:water 55:10:35.)

HPLC VARIABLES
Guard column: 50 × 6.4 25-37 μm Whatman Co-Pell ODS
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: A MeOH:200 mM ammonium acetate buffer:water 55:10:35; B MeOH:200 mM ammonium acetate buffer:water 20:10:70
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 3.1 (mobile phase A), 7.20 (2-isomer, mobile phase B), 8.55 (5-isomer, mobile phase B), 46.2 (dinitrate, mobile phase B)
Internal standard: nitroglycerin (8, mobile phase A)

OTHER SUBSTANCES
Simultaneous: saccharin, isosorbide dinitrate, pentaerythritol tetranitrate

KEY WORDS
tablets; capsules

REFERENCE
Carlson,M.; Thompson,R.D.; Snell,R.P. Determination of isosorbide dinitrate in pharmaceutical products by HPLC, *J.Chromatogr.Sci.*, **1988**, *26*, 574–578.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 10 mm long reversed-phase pellicular (Chrompack)
Column: 250 × 4.6 10 μm LiChrosorb RP-8
Mobile phase: MeCN:MeOH:buffer 16:8:76 (Buffer was 10 mL triethylamine in 760 mL water adjusted to pH 6.8 with acetic acid.)
Flow rate: 2
Injection volume: 175
Detector: UV 230

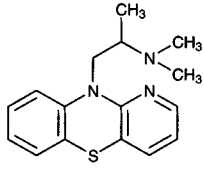
CHROMATOGRAM
Retention time: 3.6 (5 isomer)

OTHER SUBSTANCES
Simultaneous: acenocoumarol, acetaminophen, alizapride, alpiropride, amisulpride, aspirin, caffeine, carbamazepine, clonazepam, codeine, metoclopramide, nitrazepam, nitrofurantoin, theophylline
Noninterfering: amitriptyline, cisplatin, furosemide, indomethacin, isosorbide dinitrate, orphenadrine, propranolol

KEY WORDS
plasma; SPE

REFERENCE
de Jong,A.P.; Wittebrood,A.J.; du Châtinier,W.M.; Bron,J. Liquid chromatographic analysis of alizapride and metoclopramide in human plasma and urine using solid-phase extraction, *J.Chromatogr.*, **1987**, *419*, 233–242.

Isothipendyl



Molecular formula: $C_{16}H_{19}N_3S$
Molecular weight: 285.41
CAS Registry No.: 482-15-5, 1225-60-1 (HCl)
Merck Index: 5248
Lednicer No.: 1 430

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 248.8

CHROMATOGRAM
Retention time: 13.467

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 4.4

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, qui-

nine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: k' 16.22

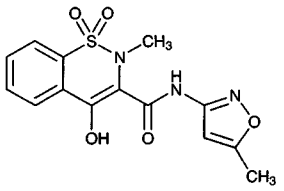
OTHER SUBSTANCES

Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE

Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

Isoxicam



Molecular formula: $C_{14}H_{13}N_3O_5S$
Molecular weight: 335.34
CAS Registry No.: 34552-84-6
Merck Index: 5258
Lednicer No.: 2 394

SAMPLE

Matrix: bile, blood, urine
Sample preparation: Plasma. 500 μL Plasma + 250 μL 1 M HCl + 5 mL diethyl ether, shake at 240 rpm on an orbital shaker for 6 min, centrifuge at 900 g at 4° for 10 min. Remove the organic phase and evaporate it to dryness at 37° under nitrogen. Reconstitute the residue in 250 μL MeCN:water 1:1, vortex for 1 min, inject a 50 μL aliquot. Urine, bile. 500 μL Urine or bile + 250 μL 1 M HCl + 5 mL diethyl ether, shake at 240 rpm on an orbital shaker for 6 min, centrifuge at 900 g at 4° for 10 min. Remove the organic phase, wash it with 2 mL pH 4.9 citric acid-phosphate buffer, and evaporate it to dryness at 37° under nitrogen. Reconstitute the residue in 250 μL MeCN:water 1:1, vortex for 1 min, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: Techsil C10 CN guard column

Column: 200 × 3.9 10 μm Techsil C10 CN (HPLC Technology)
Mobile phase: MeCN:water 22:78 (10:90 for bile analyses) containing 50 mM NaH_2PO_4, final pH 3.5
Flow rate: 2.5
Injection volume: 50
Detector: UV 365

CHROMATOGRAM
Retention time: 7.6, 17 (for bile)
Internal standard: isoxicam

OTHER SUBSTANCES
Simultaneous: piroxicam, 5-hydroxypiroxicam

KEY WORDS
plasma; isoxicam is IS

REFERENCE
Milligan,P.A. Determination of piroxicam and its major metabolites in the plasma, urine and bile of humans by high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *576*, 121–128.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Serum + 20 μL 100 mM pH 4.8 citrate buffer + 5 μL MeOH + 3 mL dichloromethane, shake for 45 s, centrifuge at 4000 rpm. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase, mix for 10 s, centrifuge, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 7 μm Separon SGX CN
Mobile phase: MeCN:10 mM phosphoric acid 70:30
Flow rate: 0.4
Injection volume: 20
Detector: UV 350

CHROMATOGRAM
Retention time: 2.74
Internal standard: isoxicam

OTHER SUBSTANCES
Extracted: piroxicam

KEY WORDS
serum; isoxicam is IS

REFERENCE
Migulla,H.; Alken,R.G.; Hüller,H. Mikromethode zur Bestimmung der Piroxicamkonzentration im Serum [Micromethod for the determination of piroxicam concentration in serum], *Pharmazie*, **1988**, *43*, 866–867.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL MeOH + 200 μL 1 M HCl, vortex at slow speed for 30 s, add 10 mL dichloromethane, shake vigorously for 30 s, centrifuge at 2500 rpm for 5 min. Remove the organic phase and add it to 20 mg anhydrous sodium sulfate, filter, evaporate to dryness under a stream of nitrogen at 35°, reconstitute the residue in 200 μL MeOH, vortex for 30 s, centrifuge at 15000 g for 5 min, inject 20 μL of the supernatant.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher 60 RP-Select B
Mobile phase: MeOH:water:acetic acid 48:45:7, pH 2.47

Flow rate: 1.1
Injection volume: 20
Detector: UV 340

CHROMATOGRAM
Retention time: 9.75
Internal standard: isoxicam

OTHER SUBSTANCES
Extracted: piroxicam
Simultaneous: droxicam

KEY WORDS
protect from light; plasma; isoxicam is IS

REFERENCE
Maya,M.T.; Pais,J.P.; Morais,J.A. A rapid method for the determination of piroxicam in plasma by high-performance liquid chromatography, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 319–322.

SAMPLE
Matrix: blood, tissue
Sample preparation: 1 mL Plasma or tissue + 700 mg potassium carbonate + 1 mL THF + 500 μL EtOH, vortex for 30 s, centrifuge at 2000 g for 5 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 70°. Reconstitute residue in 100 μL THF, vortex for 5 s, filter (0.5 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 3 μm Novapak C18
Mobile phase: THF:water 45:55 with 1% acetic acid and 5 mM 1-heptanesulfonic acid (PIC B-7, Waters)
Flow rate: 0.7
Injection volume: 20
Detector: UV 313

CHROMATOGRAM
Retention time: 8.0
Internal standard: isoxicam
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: piroxicam

KEY WORDS
plasma; rat; skin; muscle; isoxicam is IS

REFERENCE
Cerretani,D.; Micheli,L.; Fiaschi,A.I.; Giorgi,G. Rapid and sensitive determination of piroxicam in rat plasma, muscle and skin by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *614*, 103–108.

SAMPLE
Matrix: blood, tissue
Sample preparation: 1 mL Plasma or tissue + 700 mg potassium carbonate + 1 mL THF + 500 μL EtOH, vortex for 30 s, centrifuge at 2000 g for 5 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 70°. Reconstitute residue in 100 μL THF, vortex for 5 s, filter (0.5 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 3 μm Novapak C18
Mobile phase: THF:water 45:55 with 1% acetic acid and 5 mM 1-heptanesulfonic acid (PIC B-7, Waters)

Flow rate: 0.7
Injection volume: 20
Detector: UV 313

CHROMATOGRAM
Retention time: 8.0
Internal standard: isoxicam
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: piroxicam

KEY WORDS
plasma; rat; skin; muscle; isoxicam is IS

REFERENCE
Jin,L.; Lau,C.E. Determination of alprazolam and its major metabolites in serum microsamples by high-performance liquid chromatography and its application to pharmacokinetics in rats, *J.Chromatogr.B*, **1994**, *654*, 77–83.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 500 μL Plasma + 500 μL water + 100 μL THF + 250 μL 200 mM citric acid + 1 mL 2 μg/mL piroxicam in toluene, shake for 10 min at moderate speed, centrifuge at 2000 g for 5 min. Remove 600 μL of the toluene layer and evaporate it to dryness under a stream of air at 67°, reconstitute the residue in 300 μL THF, inject a 30 μL aliquot. Urine. 1 mL Urine + 100 μL THF + 100 μL 1 M HCl + 3 mL 600 ng/mL PD 79,703 in toluene, shake for 10 min, centrifuge at 2000 g for 5 min. Remove 2 mL of the toluene layer and evaporate it to dryness under a stream of air at 67°, reconstitute the residue in 300 μL THF, inject a 30 μL aliquot.

HPLC VARIABLES
Guard column: 40 × 2 Corasil C18
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: THF:water:glacial acetic acid 45:54:1 containing 5 mM 1-heptanesulfonic acid (plasma) or MeCN:water:glacial acetic acid 50:49:1 containing 5 mM 1-heptanesulfonic acid (urine)
Flow rate: 1.5
Injection volume: 30
Detector: UV 320

CHROMATOGRAM
Retention time: 8.1 (plasma), 6 (urine)
Internal standard: piroxicam (4.1), 4-hydroxy-2-methyl-2H-1,2-benzothiazine-3-carboxanilide 1,1-dioxide (PD 79,703) (14)
Limit of detection: 70 ng/mL (urine), 120 ng/mL (plasma)

KEY WORDS
plasma

REFERENCE
Daftsios,A.C.; Johnson,E.L.; Keeley,F.J.; Gryczko,C.; Rawski,V. High-performance liquid chromatographic analysis of isoxicam in human plasma and urine, *J.Chromatogr.*, **1984**, *305*, 145–151.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 100 μL Plasma + 50 μL 100 μg/mL piroxicam in MeCN + 300 μL MeCN, vortex vigorously for 1 min, centrifuge at 2000 g for 2 min, inject a 20 μL aliquot of the supernatant. Urine. 1 mL Urine + 50 μL 40 μg/mL diazepam in MeCN + 100 μL saturated KH_2PO_4 adjusted to pH 2.4 with orthophosphoric acid + 3 mL dichloromethane, vortex for 2 min, centrifuge at 2000 g for 2 min. Remove the organic layer and evaporate it to dryness

under a stream of nitrogen at 50°, reconstitute the residue in 50 μL MeCN, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 10 μm LiChrosorb RP18 ODS
Mobile phase: MeCN:buffer 50:50 (plasma) or 45:55 (urine) (Buffer was 50 mM KH_2PO_4 adjusted to pH 3.0 with orthophosphoric acid.)
Flow rate: 2
Injection volume: 20
Detector: UV 325

CHROMATOGRAM
Retention time: 3.1 (plasma), 3.8 (urine)
Internal standard: piroxicam (2.4), diazepam (5.8)
Limit of detection: 20 ng/mL (urine), 200 ng/mL (plasma)

OTHER SUBSTANCES
Noninterfering: allopurinol, cephalexin, chlorothiazide, digoxin, doxepin, furosemide, hydralazine, hydrochlorothiazide, imipramine, labetalol, mepenzolate, methyldopa, metoprolol, minoxidil, naproxen, prazosin, propranolol, sulfamethoxazole, sulfinpyrazone, trifluoperazine, trimethoprim

KEY WORDS
plasma

REFERENCE
Bury,R.W. Liquid chromatographic assay of isoxicam in human plasma and urine, *J.Chromatogr.*, **1985**, *337*, 156–159.

SAMPLE
Matrix: blood, urine
Sample preparation: 500 μL Plasma or urine + 1.1 mL 100 mM NaOH + 250 μL 1 M HCl + 5 mL diethyl ether, shake mechanically for 5 min, centrifuge at 1150 g at 4° for 5 min. Remove the ether layer and evaporate it to dryness at 35° under a stream of nitrogen. Reconstitute the residue in 250 μL 50 mM TRIS, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 10 μm μBondapak CN (plasma) or 300 × 3.9 10 μm μBondapak C18 (urine)
Mobile phase: MeCN:water 25:75 containing 50 mM NaH_2PO_4, final pH 3.2 (plasma) or THF:5 mM sodium octylsulfonate buffer:glacial acetic acid 45:54:1 (urine)
Flow rate: 1.5
Injection volume: 50
Detector: UV 365

CHROMATOGRAM
Retention time: 6 (plasma), 7 (urine)
Internal standard: isoxicam

OTHER SUBSTANCES
Simultaneous: 5'-hydroxypiroxicam, piroxicam

KEY WORDS
plasma; isoxicam is IS; (see J.Chromatogr. 1984; 305; 145)

REFERENCE
Richardson,C.J.; Ross,S.G.; Blocka,K.L.; Verbeeck,R.K. High-performance liquid chromatographic analysis of piroxicam and its major metabolite 5'-hydroxypiroxicam in human plasma and urine, *J.Chromatogr.*, **1986**, *382*, 382–388.

SAMPLE
Matrix: microsomal incubations

Sample preparation: Condition a 6 mL Bond Elut C18 SPE cartridge with 5 mL MeOH and 5 mL water. Filter 30 mL microsomal incubation, add filtrate to SPE cartridge, elute with MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 10 μm C18 (Alltech)
Mobile phase: Gradient. MeCN:200 mM pH 6.5 ammonium acetate 10:90 for 5 min, to 20:80 over 5 min, maintain at 20:80 for 10 min, to 50:50 over 10 min.
Flow rate: 1
Detector: UV 254, UV 362

CHROMATOGRAM

Retention time: 35

OTHER SUBSTANCES

Extracted: metabolites

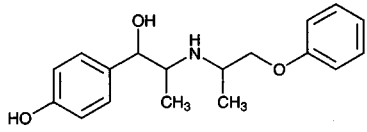
KEY WORDS

rabbit; liver; SPE

REFERENCE

Woolf,T.F.; Black,A.; Chang,T. In vitro metabolism of isoxicam by horseradish peroxidase, *Xenobiotica*, **1989**, *19*, 1369–1377.

Isoxsuprine



Molecular formula: $C_{18}H_{23}NO_3$
Molecular weight: 301.39
CAS Registry No.: 395-28-8, 579-56-6 (HCl)
Merck Index: 5259
Lednicer No.: 1 69

SAMPLE

Matrix: blood
Sample preparation: Dilute blood with an equal volume of water. 1 mL Plasma or 900 μL diluted blood + 0.9-1 mL buffer + 5 mL freshly distilled ethyl acetate, vortex for 1 min, centrifuge at 1750 g for 7 min. Remove the organic layer and evaporate it almost to dryness under a stream of nitrogen at 57°, evaporate the final 500 μL at room temperature, reconstitute the residue in 100 μL MeCN, vortex for 15 s, inject the whole amount. (Buffer was 26.5 g sodium carbonate and 21 g sodium bicarbonate in 500 mL water, pH 9.48.)

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak phenyl (plasma) or 200 × 4.6 5 μm Spheri-5 RP-18 (blood)
Mobile phase: MeCN:0.05% orthophosphoric acid 17:83 (plasma) or 63:37 (blood)
Flow rate: 2
Injection volume: 100
Detector: F ex 200 no emission filter or UV 254

CHROMATOGRAM

Retention time: 15.1 (plasma), 16.3 (blood)
Internal standard: isoxsuprine hydrochloride

OTHER SUBSTANCES

Extracted: ritodrine
Simultaneous: fenoterol